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ACTIVATION OF DEOXYCYTIDYLATE DEAMINASE BY 1-β-D-ARABINOFURANOSYLCYTOSINE-5'-TRIPHOSPHATE

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Cytosine arabinoside (1-β-D-arabinofuranosylcytosine, ara-C) has been successfully used in the treatment of certain neoplasms [1,2]. Ara-CTP, the active metabolite of ara-C, has been reported to inhibit DNA synthesis in two ways. It has been shown that ara-CTP is a competitive inhibitor of DNA polymerase (being competitive with respect to dCTP) [3] and that ara-CTP is incorporated into the growing DNA strand causing chain termination [4]. Kit et al. [5] have reported that ara-C administration caused the increase in the levels of several enzymes involved in DNA synthesis. Deoxycytidylate deaminase, which catalyzes the deamination of 5'-dCMP to 5'-dUMP, was one of the enzymes which showed increased levels of activity. Deoxycytidylate has been shown to be an important precursor of deoxythymidylate in several tissues and tumor cell lines [6-8] since the product of the reaction, dUMP, is the substrate for thymidylate synthetase. The enzyme, dCMP deaminase, has been characterized from several mammalian tissues [9-12]. The enzyme shows allosteric kinetics with respect to its substrate, dCMP. dCTP is a positive effector of the deaminase while dTTP is a negative effector. The inhibition of dCMP deaminase by dTTP can be overcome by dCTP.

Because of the competitive nature of ara-CTP with dCTP in the DNA polymerase reaction, we investigated the effect of ara-CTP on dCMP deaminase. In this report we present data to show that ara-CTP, which does accumulate in sensitive cells as a result of ara-C administration, can serve as an activator of dCMP deaminase.

The Ehrlich tumor cells were grown in mice (ICR) by weekly inoculation of recipient mice with 0.2 cc of tumor cells as taken from the mice. The mice were purchased from Lab Supply Co., Indianapolis, Indiana. The crude cell-free extract was prepared as previously described [13]. The assay procedure was essentially that reported by Maley and Maley [14]. The assay mixture for dCMP deaminase contained in a final volume of 0.2 ml: [^{3}H]-dCMP (0.05μCi, at various concentrations) and cell-free extract containing 300-500 μg protein. Heated controls were run as blanks. All assays were carried out in triplicate. Normally, reactions were run for 15 min at 37° and stopped by heating in a boiling water bath. Water (0.8 ml) was added to each sample. The sample was then put over a Dowex-50, H^+-form column (a Pasteur pipette served as the column) and the column washed with an additional 3.0 ml of 0.05 M HCl. An aliquot (1 ml) of the effluent was taken for measurement of radioactivity by liquid scintillation counting.

High pressure liquid chromatography was carried out on a Varian 4200 gradient liquid chromatograph with a linear gradient of 0.01 M ammonium phosphate, pH 2.78 to 0.5 M ammonium phosphate, pH 4.82 buffer. A Whatman Partisil SAX column (25 X 0.46 cm) was used. The flow rate was 2 ml/min. The gradient change was 5%/min.

The nucleotide compounds used in these studies were purchased from Sigma Chemical Company. The [^{3}H]dCMP, 27 and 25.1 Ci/mmole, was purchased from Amersham Searle and New England Nuclear respectively. The [^{3}H]dCMP was purified on Dowex-50, H^+ before use.

The dCMP deaminase activity in the crude cell-free extracts prepared from Ehrlich cells showed sigmoidal kinetics with respect to dCMP as shown in Fig. 1. The addition of dCTP or dCDP markedly activated the dCMP deaminase and also altered the kinetics to the typical hyperbolic kinetics. Under the conditions used, the dCMP deaminase assay was linear with time over a 30 min period. This linearity with time was observed either at low or high substrate concentration or at low substrate concentration in the presence and absence of dCTP. The presence of dCTP did not extend the linearity of the reaction beyond that in the absence of dCTP.

Fig. 1 also shows the effect of dCTP and dCDP concentrations on the allosteric activation of the dCMP deaminase. dCTP, on a concentration basis was more stimulatory to the deaminase activity than was the dCDP. dTTP inhibited the deaminase, but the inhibition could be reversed by either dCTP or dCDP.

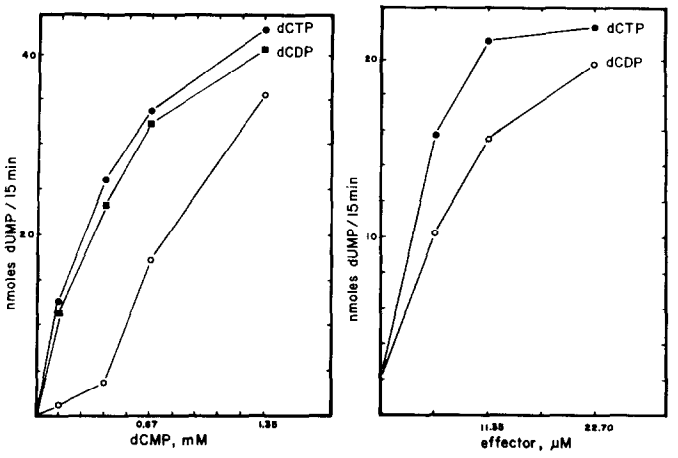

Fig. 1. dCMP deaminase activity as a function of substrate concentration in the presence and absence of the positive effectors and as a function of dCTP and dCDP concentration. In the left panel, the concentration of dCTP was 0.11 mM; the concentration of dCDP was 0.16 mM. The deaminase activity in the absence of effector is shown by O———O. In the right panel, the substrate concentration was 0.2 mM. The deaminase activity in the absence of effector was 2 nmoles dUMP/15 min.

The activation of dCMP deaminase by dCDP was further studied to determine if dCDP was a positive effector or in fact, was converted to dCTP in a cell-free extract. To test this the standard assays were set up with dCDP or dCTP as activator. After termination of the reaction, aliquots of the reaction mixture were analyzed by high pressure liquid chromatography. dCMP, dCDP and dCTP standards eluted from the column at 2.1, 8.9 and 19.8 min respectively. In the samples with dCDP as activator, only dCMP and dCDP were observed, while in the sample with dCTP as activator, only dCMP and dCTP were observed. These results indicated that dCDP can serve as an activator of dCMP deaminase.

Since ara-CTP inhibits DNA polymerase, presumably as an analog of dCTP, the effect of ara-CTP on dCMP deaminase activity was studied. As seen in Fig. 2, ara-CTP was a positive effector of dCMP deaminase. To reach the same level of activation of dCMP deaminase as we obtained with dCTP, approximately 10 times the concentration of ara-CTP was required. However, at lower levels of ara-CTP, there was significant activation. Ara-CTP stimulated the dCMP deaminase activity at all concentrations of dCMP tested in a manner similar to the data seen in Fig. 1. The inhibition of dCMP deaminase activity by dTTP was overcome by ara-CTP, indicating that ara-CTP was serving as a dCTP analog in the deaminase reaction.

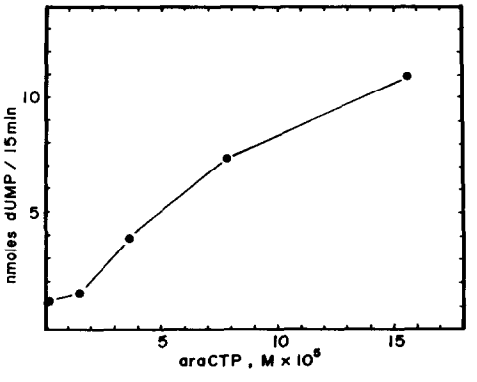

Fig. 2. dCMP deaminase activity with ara-CTP as the positive effector. The substrate concentration was 0.2 mM.

Deoxycytidylate deaminase has been shown to be elevated in rapidly growing tissues such as regenerating liver and tumor tissue [10]. The level of enzyme activity has been shown to correlate with tumor growth rate [11]. The importance of this enzyme to DNA synthesis has been further implicated by studies which showed that deoxycytidine contributed a large percentage of the dUMP or thymidine in phytohemagglutinin-stimulated human lymphocytes, HeLa cells and hepatoma cells [6-8]. These results showed that dCMP deaminase does function in the intact cell to provide much of the substrate (dUMP) for the thymidylate synthetase reaction.

These current studies show that ara-CTP, the active metabolite of ara-C, is a relatively strong positive effector of dCMP deaminase. Studies have shown that recovery of DNA synthesis after ara-C administration is quite slow and is dependent on the concentration of ara-C given [15]. In addition, a correlation has been shown between the retention of ara-CTP and the response to therapy [16]. These results would indicate that ara-CTP must accumulate in the cells which are sensitive to ara-C. A possible effect, therefore, of ara-C administration would be to stimulate the dCMP deaminase activity, which in turn would increase the level of dUMP in the treated cells. Increasing the dUMP level would have the effect of increasing the dTMP pools and in turn dTTP levels. dTTP has been shown to be an activator of GDP reductase, and an effective inhibitor of CDP reductase [17], and thymidine kinase [18] in addition to dCMP deaminase. The data suggest that other possible effects of ara-C may be due to the activation of dCMP deaminase which in turn could cause an overall alteration in deoxyribonucleotide formation and interconversions leading to the inhibition of DNA synthesis.

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